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CD300A AND CD300C ON PLASMACYTOID DENDRITIC CELLS ARE DOWN-REGULATED BY TLR7 AND TLR9 LIGAND INDUCED TYPE I INTERFERON

Hart, D.N.J.[1], Clark, G.[1], Ju, X.[1], Zenke, M.[2]. [1] Mater Medical Research Institute, Brisbane, Queensland, Australia; [2] Institute for Biomedical Engineering, Aachen, Germany.

Plasmacytoid dendritic cells (pDC) constitute a distinct population of DC in the peripheral and secondary lymphoid organs. After activation with the ligands for Toll-like receptors (TLRs), particularly TLR7 and TLR9, pDC secrete large amounts of type I interferons and differentiate into mature DC, expressing co-stimulatory molecules and other cytokines. New immunoregulatory molecules on human pDC were sought and their regulation by TLR ligands assessed by pDC gene expression profiling. We established that both the CD300a and CD300c cell surface molecules are expressed by pDC. Because CD300 molecules play important roles in regulating immune responses, we investigated their contribution to pDC function in more detail. First, their down-regulation by CpG-ODN was confirmed by real time PCR and RT-PCR for CD300a and CD300c. Using the monoclonal antibody CMRF-35, to detect both CD300a and CD300c, we demonstrated that both TLR7 and TLR9 ligands down-regulated cell surface CD300a and CD300c. We showed that exogenous IFN-α down-regulated CD300a/c expression in pDC suggesting that the TLR ligands induced down-regulation of CD300a/c might be an indirect effect. In subsequent experiments, we added neutralizing antibody to IFN-α and found that it abolished the CpG-ODN induced CD300a/c down-regulation.

The effect of CD300a and CD300c activation on the function of pDC was investigated by cross-linking CMRF-35 on pDC. This had no effect on CD80, CD83 and CD86 expression, but decreased MHC-II expression. Significant reductions in pDC TNF-α and IL-6 production occurred after CMRF-35 cross-linking, however, in marked contrast, CpG-ODN induced IFN was increased in these experiments. Thus the immune regulators CD300a and CD300c regulate the cytokine production in pDC, whilst the pDC released cytokines regulated the CD300a/c expression. This suggests that CD300 molecules may play a pivotal role in fine tuning pDC driven immune responses and may contribute to the pDC biology of allogeneic bone marrow transplantation.

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RECENT TRENDS IN UNRELATED DONOR STEM CELL TRANSPLANTATION: A REPORT FROM THE ABMTRR

Nivison-Smith, I.[1], Bradstock, K.F.[2], Dodds, A.J.[3], Ma, D.D.F.[3], Szer, J.[4]. [1] ABMTRR, Darlinghurst, NSW, Australia; [2] Westmead Hospital, Westmead, NSW, Australia; [3] St Vincent's Hospital, Darlinghurst, NSW, Australia; [4] Royal Melbourne Hospital, Parkville, Victoria, Australia.

Aim: To investigate and highlight recent trends among patients undergoing allogeneic haematopoietic stem cell transplant (HSCT) with unrelated donors (URD) in Australia and New Zealand. **Methods:** Patients were selected from the database of the Australasian Bone Marrow Transplant Recipient Registry (ABMTRR). Patients selected for this study had undergone HSCT with URD between the years of 2001 and 2006. **Results:** A total of 891 allogeneic HSCT with URD were performed in the years 2001 to 2006. The annual numbers have increased steadily, from 101 in 2001 to 172 in 2005 and 146 in 2006. A total of 257 HSCT (29%) involved patients aged up to 15 years, and 634 (71%) involved patients aged 16 or over. Among paediatric transplants, 128 (50%) utilised cord blood (including double cord), 96 (37%) utilised bone marrow and 33 (13%), peripheral blood. The major indication for transplant in paediatric HSCT was ALL (79, 31% of paediatric HSCT). Among adult transplants, the stem cell source was peripheral blood in 407 (64%), marrow in 188 (30%) and cord blood in 39 (6%). The major indication for adult transplant was AML (228, 36% of adult HSCT). The number of adult HSCT for patients aged 50 years or over increased from 20 in 2001 to 39 in 2006. The number of adult HSCT involving reduced intensity conditioning also increased, from 11 in 2001 to 48 in 2005 and 30 in 2006. **Conclusions:** The annual numbers of URD HSCT in Australasia have increased steadily in recent years. Recent trends in practice include increases in numbers of older patients and HSCT using reduced intensity conditioning. The ABMTRR is a valuable national resource which provides accurate and timely information on HSCT activity and outcome in these two countries.

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EFFICACY AND TOXICITY OF A CONDITIONING REGIMEN WITH 8-GY TOTAL BODY IRRADIATION, FLUDARABINE AND CYCLOPHOSPHAMIDE FOR ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PEDIATRIC HEMATOLOGIC MALIGNANCIES

Yanagisawa, R.[1], Nakazawa, Y.[1], Sakashita, K.[1], Tanaka, M.[1], Kamijo, T.[2], Shiohara, M.[1], Koike, K.[1]. [1] Shinshu University School of Medicine, Matsumoto, Japan; [2] Chiba Cancer Center Research Institute, Chiba, Japan.

We examined the efficacy and toxicity of a conditioning regimen with fractionated 8-Gy total body irradiation, fludarabine, and cyclophosphamide in allogeneic hematopoietic stem cell transplantation (HSCT) for pediatric hematologic malignancies. Among a total of 22 children who received related or unrelated HSCT, nine were transplanted with refractory disease and/or from HLA 2 or more-loci mismatched family donors. The Seattle grading system revealed that 18 patients had no regimen-related toxicity, whereas the remaining patients had grade I gastrointestinal toxicity alone. According to the National Cancer Institute Common Toxicity Criteria, Grade II or higher toxicity in the liver, mucosa, and gastrointestinal tract among eight organs was documented in approximately 10–35% of patients, and was attributed to engraftment syndrome and/or acute graft-versus-host disease. None of the patients developed graft failure. The estimated overall survival and leukemia-free survival (LFS) at 2 years were 56.3% and 46.7%, respectively, in 10 patients with acute lymphoblastic leukemia; 91.7% and 81.5%, respectively, in 12 patients with myeloid leukemia. The incidence of treatment-related mortality was 5.3% at 2 years. It is possible that our preparative regimen confers successful engraftment combined with minimized regimen-related toxicity, and a favorable LFS rate for children with hematologic malignancies, especially for those with myeloid leukemia.

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ROLE OF RITUXIMAB FOR PROPHYLACTIC OR PREEMPTIVE THERAPY OF EBV-DNA-emia AND THERAPY OF EBV-PTLD IN HSCT RECIPIENTS: A SYSTEMATIC REVIEW

Styczynski, J.[1], Einsele, H.[2], de la Camara, R.[3], Engelhard, D.[4], Reusser, P.[5], Ward, K.N.[6], Ljungman, P.[7]. [1] Collegium Medicum UMK, Bydgoszcz, Poland; [2] University Hospital, Wuerzburg, Germany; [3] Hospital de la Princesa, Madrid, Spain; [4] Hadassah University Hospital, Jerusalem, Israel; [5] Basel University School of Medicine, Porrentruy, Switzerland; [6] University College London, London, United Kingdom; [7] Karolinska University Hospital, Stockholm, Sweden.

Objective: To evaluate the role of rituximab for prevention and treatment of posttransplant lymphoproliferative disorder (EBV-PTLD) in HSCT recipients. DATA SOURCES: A PubMed search (1966-September 2007) was conducted using the key words: EBV, posttransplant lymphoproliferative disorder, stem cell transplantation, rituximab. References of relevant articles and abstracts from recent hematology and stem cell transplantation scientific meetings (2003–2007) were also reviewed. STUDY SELECTION AND DATA EXTRACTION: Prospective and retrospective studies identified from the data sources were evaluated, and all data deemed relevant were included in this analysis. DEFINITIONS: Prophylaxis of EBV-DNA-emia (EBV reactivation) – rituximab given to seropositive, EBV-DNA-negative patient (or when donor was seropositive) to prevent EBV reactivation. Preemptive therapy – rituximab given to an asymptomatic patient with EBV detected by a screening assay. Treatment of PTLD – rituximab applied to a patient with an overt EBV-PTLD. **Results:** High risk HSCT for PTLD development were allogeneic HSCT with following risk factors: unrelated/mismatch HSCT; T-cell depletion or ATG/OKT3 use; EBV serology mismatch; primary EBV infection; splenectomy. The risk increased with the number of risk factors. In addition to quantification of EBV DNA load, analysis of the level of EBV-specific T cell reconstitution during EBV reactivation might be an

important second parameter to define patients at risk for EBV-PTLD who might be candidates for preemptive interventions. Total number of 49 papers and 19 conference abstracts were available. Most reports included only a small number of patients. Overall response rates were: 61% (14/23 pts) for prophylactic use of rituximab; 88% (214/243 pts) for preemptive therapy with rituximab, and 72% (56/77 pts) for rituximab used for therapy of EBV-PTLD disease. Rituximab was safe and well tolerated. **Conclusions:** Since a significant proportion of patients died from PTLD in spite of rituximab treatment, and since availability of EBV-CTLs is limited, it is reasonable to recommend preemptive treatment with rituximab for increasing EBV-DNA-emia. Emerging problem might be related to down-regulation of CD20 expression on PTLD cells following repeated therapy, causing refractoriness to rituximab treatment due to lack of CD20 expression. Further studies are needed to confirm the efficacy of these treatment strategies.

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CHRONIC GRAFT VERSUS HOST DISEASE AND PRETRANSPLANT DISEASE STATUS PREDICT OUTCOMES IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES UNDERGOING REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION

Shah, M., Agha, M., Mapara, M., Lenhart, K., Raptis, A. University of Pittsburgh Cancer Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA.

Reduced intensity stem cell transplantation (SCT) is an effective treatment modality for patients with hematologic malignancies who are not candidates for conventional myeloablative SCT. We conducted a retrospective review of all patients with hematologic malignancies receiving a reduced intensity allogeneic SCT from July 2002 to July 2007. Data pertaining to patient demographics, engraftment, disease status pre/post transplant, graft versus host disease (GVHD), and HLA matching was analyzed to identify factors significantly affecting the clinical outcome.

73 patients, with a median age of 55 (range of 19–70) and with the diagnoses of ALL (n = 8), AML (n = 30), CLL (n = 3), CML (n = 1), Hodgkins (n = 7), non-Hodgkins (n = 7), MDS (n = 11), and MM (n = 6) underwent a reduced intensity SCT using a fludarabine based conditioning regimen. Median and mean follow-up times were 5.6 and 9.9 months respectively. 39 (53%) received unrelated donor grafts and 34 (47%) received sibling donor grafts. 56 patients (77%) received fully matched grafts whereas 17 patients (23%) had an antigen or allele mismatch. Neither of those variables affected clinical outcome. Median time to neutrophil recovery was 15 days (range of 9–41) and median time to platelet recovery was 18 days (range of 9–42). Graft failure was observed in 6 of the 73 patients. Median overall survival and disease free survival for all patients was 7.7 and 6.6 months. Median overall survival for patients with persistent disease or in remission at the time of the SCT was 5.6 and 21.8 months ($p = 0.01$) while that for disease free survival was 5.7 and 8.4 months ($p = 0.06$). Median overall survival with and without chronic GVHD was 25.6 and 9.4 months ($p < 0.0001$) while median disease free survival was 18.2 and 6.0 months ($p < 0.0001$). Patients with limited chronic GVHD have not yet reached median overall survival while median disease free survival was 18.4 months. Those with extensive chronic GVHD had medians of 9.2 months for both overall and disease free survival ($p = 0.004$ and $p = 0.02$).

Reduced intensity SCT is an effective treatment modality in patients with hematologic malignancies, though it is most effective in patients who are in remission at the time of transplant and should be offered in this setting. Patients with limited chronic GVHD had a better outcome suggesting the presence of potent anti-tumor activity of the donor immune competent cells without the detrimental effects in clinical outcome caused by extensive chronic GVHD.

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THE FEASIBILITY OF USING CCR5Δ32/Δ32 HEMATOPOIETIC STEM CELL TRANSPLANTS FOR IMMUNE RECONSTITUTION IN HIV-INFECTED CHILDREN

Chen, T.K.[1], Moore, T.B.[2], Territo, M.[3], Chow, R.[4], Tonai, R.[4], Petz, L.[4], Rossi, J.[5], Mitsuyasu, R.[3], Rosenthal, J.[6], Forman, S.[6], Zaia, J.A.[5], Bryson, Y.J.[1]. [1] David Geffen School of Medicine at UCLA, Los Angeles, CA; [2] David Geffen School of Medicine at UCLA, Los Angeles, CA; [3] David Geffen School of Medicine at UCLA, Los Angeles, CA; [4] StemCyte, Inc., Covina, CA; [5] Beckman Research Institute of City of Hope, Duarte, CA; [6] Beckman Research Institute of City of Hope, Duarte, CA.

Despite treatment with highly-active antiretroviral therapy (HAART), some HIV infected patients fail to reconstitute their immune functions and are intolerant to side effects of long term HAART, indicating the need to develop novel immune-based therapies. Hematopoietic stem cells (HSC) have been used to reconstitute the immune system in patients with primary immunodeficiencies, genetic diseases, and childhood cancers. Transplantation for HIV infection has been attempted, but re-infection of the grafted cells is a concern. Studies demonstrate that homozygosity for a 32 bp deletion in the HIV-1 CCR5 co-receptor gene (CCR5Δ32) is associated with protection against R5 HIV infection, while CCR5Δ32 heterozygosity is associated with slower disease progression. Thus, T-cells developing after a transplant with HSC homozygous for the CCR5Δ32 mutation should be resistant to R5 HIV infection. The CCR5Δ32 allele is found in Caucasian populations with 1% being homozygous and 15–20% heterozygous. We have shown that the HIV coreceptor tropism may revert from X4 to R5 under the pressure of HAART. In addition, we and others have found that the replication competent virus recovered from CD4 T-cells reservoirs in children are R5. We evaluated the feasibility of HSC transplantation using CCR5Δ32/Δ32 stem cells for the immune reconstitution of HIV-infected children who are immunocompromised despite HAART. StemCyte, an international cord blood bank, has been actively screening their cord blood units (CBUs) for the CCR5Δ32 allele. To date, StemCyte has identified 30 homozygotes and 754 heterozygotes for the CCR5Δ32 mutation among 10,488 CBUs screened. 44 patients were randomly selected from our cohort of HIV-infected children and adolescents. HLA typing was performed to identify patients with potential HLA matched CBUs. Initial statistical considerations demonstrated that 100–150 patients would need to be screened in order to identify 2–4 potential HLA matched CBUs. We found 7 (15.9%) of our patients with a 4/6 HLA match to the identified homozygous CBUs, and 20 (45.4%) of our patients that are a 3/6 match to the identified homozygous CBUs. 2 of 7 patients with an identified 4/6 HLA match continue to have detectable plasma viremia despite HAART. 2 of 7 patients have cords with sufficient cell count for transplant. Thus, we identified and characterized 7 potential HIV-positive patients who could benefit from a transplant of HLA-matched, CCR5Δ32/Δ32 umbilical cord blood stem cells.

HLA matched (4 out of 6) patients and homozygous CCR5Δ32 cord blood units

Patient			Cord Blood Unit				
Age	Gender	Weight (kg)	Gender	Volume (mL)	TNC/kg ($\times 10^7$)	CD34/kg ($\times 10^5$)	Mismatched loci
17 years	M	70.1					
			M	95.0	0.61	0.18	B
			M	64.68	0.81	0.26	B, DRB1
			F	64.20	1.07	0.18	B, DRB1
			M	67.00	1.50	0.21	A, DRB1
11 years	M	41.3					
			F	64.20	1.82	0.30	A, B
5 years	M	23.2					
			M	64.68	2.46	0.78	A, B
25 years	M	60.9					
			F	152.20	5.77	2.17	B, DRB1
24 years	M	70.4					
			M	64.29	0.97	0.14	A, DRB1
11 years	F	42.6					
			F	63.80	1.55	0.31	A, B
22 months	M	12.7					
			F	64.20	5.92	0.97	A, B